Inhibition of calcium currents in cultured rat dorsal root ganglion neurones by (−)-baclofen

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1 Voltage-dependent inward calcium currents (I_{Ca}) activated in cultured rat dorsal root ganglion neurones were reversibly reduced in a dose-dependent manner by (−)-baclofen (10 μM to 100 μM).

2 Baclofen (100 μM) reduced the calcium-dependent slow outward potassium current (I_{K(Ca)}). This current was abolished in calcium-free medium and by 300 μM cadmium chloride. The action of baclofen on I_{K(Ca)} was reduced when the calcium concentration in the medium was increased from 5 mM to 30 mM.

3 The calcium independent fast transient voltage-dependent outward current (I_{K(VT)}) was also reduced by baclofen; this effect remained present when Ca^{2+}-free medium was used to prevent contamination by I_{K(Ca)^1}.

4 4-Aminopyridine (500 μM) reduced I_{K(VT)} and induced a small increase in I_{Ca}. The action of baclofen on I_{Ca} was partially antagonized by 4-aminopyridine.

5 GABA_b receptor-mediated inhibition of I_{Ca} in cultured rat dorsal root ganglion neurones involves a direct mechanism rather than resulting indirectly from an increase in the residual outward potassium currents activated by depolarization. The reduction in I_{Ca} by baclofen was variable and dependent on the amplitude of control I_{Ca}, larger currents being more resistant to the baclofen-induced inhibition.

Introduction

Baclofen (β-β-chlorophenyl GABA) is a specific γ-aminobutyric acid_b (GABA_b) receptor agonist which inhibits neurotransmission at several peripheral and central synapses including primary afferent terminals in the spinal cord (Fox et al., 1978). Both presynaptic and postsynaptic GABA_b receptor-mediated inhibition has been identified (Bowery et al., 1980; Newberry & Nicoll, 1985). GABA, like a number of other neurotransmitters, has been found to decrease the duration of calcium-dependent action potentials in dorsal root ganglion (DRG) neurones (Dunlap & Fischbach, 1981; Desarmenien et al., 1984; Deisz & Lux, 1985). In cultured chick DRGs this appeared to be due to a direct effect of GABA on the inward calcium current rather than an enhancement of outward potassium conductances (Dunlap & Fischbach, 1981; Deisz & Lux, 1985). This response to GABA is bicuculline-resistant, and appears to be mediated by GABA_b receptor activation, although Dunlap (1984) has shown that bicuculline- and muscimol-sensitive GABA_A receptors which mediate increases in chloride

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Figure 1 The dose-response relationship for inhibition of the inward calcium current (I_{Ca}) by baclofen. For each cell, the percentage reduction of the peak amplitude of the maximum I_{Ca} was determined following leakage current subtraction. Each cell was exposed to only one concentration of baclofen. For each dose of baclofen used, the mean percentage reduction is given; vertical lines show s.e.mean (n = 5; except 100 μM, n = 11). For all cells the holding potential (V_H) was −70 mV.

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ion permeability co-exist with GABA<sub>B</sub> receptors on these sensory neurones. Baclofen offers a tool to investigate selectively the involvement of GABA<sub>B</sub> receptors in depression of voltage-dependent inward calcium currents which have been implicated in presynaptic inhibition (Dunlap & Fischbach, 1981). The role of calcium influx through voltage-activated calcium channels in transmitter release is well established (Llinas et al., 1981).

The central effects of baclofen cannot be explained entirely by inhibition of calcium currents, since in voltage-clamped hippocampal neurones, baclofen generated an outward potassium current which was inhibited by external Ba<sup>2+</sup> and by internal Cs<sup>+</sup> (Gähwiler & Brown, 1985). In addition, Désarmenien et al. (1984) failed to find a shortening of action potentials by baclofen in adult rat DRGs loaded with Cs<sup>+</sup>, suggesting that potassium currents are also involved in the action of baclofen in these cells.

**Figure 2** The reversibility of the inhibition of the inward calcium current (I<sub>Ca</sub>) by baclofen following computer subtraction of leakage current is illustrated under control conditions, in the presence of 100 μM baclofen and after partial recovery (5 min after baclofen application). The holding potential (V<sub>H</sub>) was −70 mV and the currents were activated by +70 mV step commands for 100 ms. The % reduction induced by baclofen illustrated here is 92% and the recovery is to 65% of control (1 of 11 experiments).

**Figure 3** Voltage-current relationships showing the baclofen-induced reduction in the inward calcium current (I<sub>Ca</sub>) (following leakage current subtraction). The peak amplitude of the net inward current I<sub>Ca</sub> is plotted against voltage achieved during the step command. Con (■), control; Bac (▲), in the presence of 100 μM baclofen; Rec (●), after partial recovery. V<sub>H</sub> = −70 mV.

**Figure 4** The effect of baclofen on the calcium-dependent slow outward potassium current (I<sub>K(Ca)</sub>). Depolarizing step commands of 5 s duration from a holding potential of −60 mV activated a slow outward current (I<sub>R</sub>) which developed following the ohmic current response. The responses were obtained in standard recording medium containing 2.5 μM tetrodotoxin and 5 mM CaCl<sub>2</sub> (in the absence of tetraethylammonium); patch pipettes containing KCl were used. In (a) voltage-current relationships are shown with I<sub>R</sub> plotted against the voltage achieved during the step command. Control (Con, ■), baclofen 100 μM (Bac, ▲) and cadmium 300 μM (Cd<sup>2+</sup>, ●). In (b) currents activated by +100 mV step command from a holding potential of −60 mV are shown (recorded from a different cell). I<sub>R</sub> in the control record was blocked by 100 μM baclofen revealing a small transient outward current (1 of 5 experiments).
In an initial study, we showed that baclofen reduced calcium currents in cultured neonatal rat DRGs (Dolphin et al., 1986) and this investigation has now been extended to include the effect of baclofen on both calcium and potassium currents in these cells.

Methods

Dissociated cultures of dorsal root ganglion (DRG) neurones from 2 day old rats were prepared as described by Forda & Kelly (1985). The whole cell recording technique (Hamill et al., 1981) was used to measure voltage-activated calcium and potassium currents from cells which had been maintained in culture for between 4 and 8 weeks. Patch electrodes of 4–10 MΩ resistance were used to form seals in excess of 1 GΩ onto the cell membrane before destroying the membrane patch by additional negative pressure and recording from the whole cell. The cells were voltage clamped using an Axoclamp-2 switching voltage clamp amplifier operated at a sampling rate of 10 kHz. Experiments were performed at room temperature, and currents were evoked at a frequency of 0.03 Hz to reduce frequency-dependent run-down of calcium currents. (−)-Baclofen was applied by continuous low pressure ejection (less than 1 psi) from a micropipette (tip diameter of approximately 10 μm) placed about 100 μm from the cell. This method of application has been shown to bathe the cell in the same concentration of drug as is in the pipette, to within 10% (Choi & Fischbach, 1981).

The standard recording medium contained (mM): NaCl 130, KCl 3, MgCl₂ 0.6, NaHCO₃ 1.0, HEPES 10, glucose 4 and either CaCl₂ 5 or BaCl₂ 2.5, tetrodotoxin (TTX) 2.5 μM was also present. Ba²⁺ was substituted for Ca²⁺ in experiments where voltage-dependent calcium currents were examined since Ba²⁺ is able to carry charge through Ca²⁺ channels in a number of cell types (Fenwick et al., 1982) including DRG neurones (Fedulova et al., 1985). The inward current carried by Ba²⁺ has been shown to have similar characteristics to that carried by Ca²⁺, although the current decayed more slowly and K⁺ conductance was reduced in the presence of Ba²⁺ (Fedulova et al., 1985). The term I₉Ca will therefore be used to describe this current. For measurement of I₉Ca the recording medium also contained tetraethylammonium (TEA).

![Figure 5](image)

**Figure 5** The effect of baclofen (100 μM) on the calcium-dependent slow outward potassium current (I₉Ca) recorded in medium containing 30 mM CaCl₂. The recording medium was otherwise identical to that used in the experiments shown in Figure 4. In (a) I₉ is plotted against the voltage achieved during the step command. V₉ = −60 mV. The control (Con, []) voltage–current relationship and that during application of baclofen 100 μM (Bac, ▲) are shown. In (b) (same cell) the traces illustrate the currents activated by depolarizing and hyperpolarizing step commands (20 to 80 mV) (top traces), under control conditions (middle traces) and in the presence of baclofen (bottom traces). I₉(V) was not observed in this cell (1 of 4 experiments).
bromide (25 mM). All recording media were adjusted to pH 7.4 with NaOH and to 320 mosmol by the addition of sucrose.

Patch electrodes were filled with a solution containing (mM): KCl or Cs acetate 140, EGTA 1.1, MgCl\(_2\) 2, CaCl\(_2\) 0.1 and HEPES 10. The pH was adjusted to 7.2 with KOH or CsOH and the osmolarity to 310 mosmol with sucrose. Drugs used were (−)-baclofen (Ciba-Geigy), 4-aminopyridine and TEA bromide (Sigma).

**Results**

(−)-Baclofen at concentrations between 10 μM and 100 μM reduced voltage-dependent inward calcium currents (ICA) in a dose-dependent manner (Figure 1). The action of baclofen on I\(_{Ca}\) was partially reversible at 75 μM and 100 μM (Figure 2), and completely reversible between 10 μM and 50 μM. The recovery of I\(_{Ca}\) after application of 100 μM baclofen was to 80.4 ± 3.5% (mean ± s.e. n = 7) of the initial control amplitude of I\(_{Ca}\). The dose-response relationship was generated on different cells for each application of baclofen because of the time-dependent run-down of I\(_{Ca}\). I\(_{Ca}\) was reduced by baclofen at all potentials as shown by the voltage-current plots (Figure 3), while there was no consistent or dose-dependent change in either the null potential or the voltage-dependence of the current. No consistent effect of baclofen was observed on I\(_{K}\), the current required to maintain the cells at their holding potential.

The rate of rise of the maximum I\(_{Ca}\) was reduced in the presence of baclofen. The slowing of the current was reflected by the increase in its half-time to peak, which in the presence of 100 μM baclofen increased from 4.6 ± 0.7 to 7.0 ± 1.5 ms (mean ± s.e.mean; n = 5), a mean increase of 52%.

The conditions in which I\(_{Ca}\) was measured (internal Cs\(^+\), external Ba\(^{2+}\) and TEA) were designed to minimize potassium conductances and the data thus suggest that baclofen has a direct action on I\(_{Ca}\) rather than increasing underlying voltage or Ca\(^{2+}\)-dependent potassium currents. To examine this hypothesis, the effect of baclofen on K\(^+\) conductance was also investigated. Potassium currents were recorded from DRG neurones bathed in recording medium containing 5 mM CaCl\(_2\), with KCl replacing Cs acetate in the patch solution. The currents were evoked by 5 s depolarizing voltage step commands from a holding potential of −60 mV, at a frequency of 0.03 Hz and preceded by hyperpolarizing pulses of the same amplitude. Baclofen was found markedly to reduce the slowly developing outward relaxations (I\(_{K}\)) which

![Figure 6](image)

*Figure 6* The effect of baclofen (Bac) and 4-aminopyridine (4-AP) on fast transient outward currents (I\(_{Kw}\)) recorded in 0 mM CaCl\(_2\). The recording medium was otherwise identical to that described in the legend to Figure 4. I\(_{Kw}\) was measured from the peak of the transient to the end of the decay before the step command terminates. (a and c) Illustrate the amplitude of I\(_{Kw}\) plotted against voltage achieved during the step command for a control cell (Con) and in the presence of baclofen (100 μM), a) or 4-AP (500 μM, c). (b and d) Show control outward currents activated by +100 mV step commands from a holding potential of −60 mV and the reduction in the current induced by baclofen (b) and 4-AP (d). (a and b, 1 of 5 experiments; c and d, 1 of 5 experiments).
followed the ohmic current responses to depolarizing step commands (Figure 4a,b). Under control conditions the mean amplitude of $I_R$ was 437 ± 56 pA at 0 mV and 640 ± 64 pA at +40 mV ($n = 5$). Baclofen (100 μM) reduced $I_R$ at 0 mV and +40 mV by 82.0 ± 7.6% and 79.3 ± 8.2% respectively. $I_R$ was also reduced by 300 μM cadmium chloride (Figure 4a) and was absent in calcium-free medium, where 5 mM CaCl$_2$ was substituted by 5 mM MgCl$_2$ and 0.2 mM EGTA, suggesting that it represented a calcium-dependent potassium current ($I_{K(Ca)}$). However, while the change from calcium-containing to calcium-free medium abolished $I_R$, we were unable to demonstrate its recovery or the development of calcium after initial incubation in calcium-free medium.

The amplitude of $I_{K(Ca)}$ was increased by elevating external Ca$^{2+}$ to 30 mM. During voltage step commands from -60 mV to 0 mV and +20 mV, $I_R$ was 620 ± 190 pA and 800 ± 130 pA ($n = 4$) respectively. Under these conditions the inhibition by baclofen of $I_R$ was markedly reduced (Figure 5), being 16.2 ± 3.2% and 18.7 ± 4.6% ($n = 4$) at 0 mV and +20 mV respectively, suggesting the Ca$^{2+}$ antagonizes this action of baclofen.

In some neurones, depolarizing voltage step commands induced rapidly developing slowly inactivating calcium-independent outward currents; however, these were usually contaminated by $I_{K(Ca)}$. Unlike $I_{K(Ca)}$, this transient current was resistant to TEA, but it was blocked by another potassium channel blocker 4-aminopyridine (4-AP). To study the action of baclofen on this current which we have termed $I_{K(V)}$, experiments were carried out either in calcium-free medium or in medium containing 30 mM calcium. The former condition abolished $I_{K(Ca)}$ and in the latter condition the action of baclofen on $I_{K(Ca)}$ was markedly attenuated, in both cases allowing its effect on $I_{K(V)}$ to be determined.

In the cells in which $I_{K(V)}$ was observed, it was activated by depolarizing steps of greater than 20 mV from a holding potential of −60 mV (21/35 cells). In calcium-free medium, baclofen (100 μM) and 4-AP (500 μM) reduced the peak amplitude of $I_{K(V)}$ by 35 ± 12% and 54 ± 18% ($n = 5$) respectively (Figure 6a and c). Both baclofen (Figure 6b) and 4-AP (Figure 6d) also reduced total outward current measured at the end of the depolarizing step command, by 29% and 21% respectively, although neither affected $I_H$. In 30 mM Ca$^{2+}$, $I_{K(V)}$, unlike $I_{K(Ca)}$, was still markedly reduced by baclofen (Figure 7).

The effect of 4-AP (500 μM) on $I_C$ was also investigated to determine whether $I_C$ was contaminated by a 4-AP-sensitive outward current. Despite the presence of internal Cs$^{+}$ and external TEA and Ba$^{2+}$, application of 4-AP by pressure ejection induced a small increase (15.0 ± 2.9%, $n = 5$) in $I_C$. This action of 4-AP was completely reversible (Figure 8). The effect of 4-AP on the ability of baclofen to inhibit $I_{Ca}$ was next examined. The mean reduction in $I_{Ca}$ induced by 100 μM baclofen alone was 80.0 ± 4.9% ($n = 11$). When 500 μM 4-AP was present in both the bath and the pressure ejection pipette used for applying baclofen, the effect of baclofen on $I_{Ca}$ was antagonized. The mean percentage reduction in $I_{Ca}$ induced by baclofen in the presence of 4-AP was 35.5 ± 8.8% ($n = 3$). However, continuous exposure of cells to 4-AP in the bath resulted in a run down of $I_{Ca}$. When 4-AP was present in the baclofen pipette the mean percentage reduction in $I_{Ca}$ by baclofen was 56.3 ± 5.6% ($n = 15$). Since a considerable variation was observed in the reduction of $I_{Ca}$ induced both by baclofen alone and by baclofen with 4-AP, these data

**Figure 7** The effect of baclofen on both the calcium-dependent slowly developing outward relaxation current ($I_{K(Ca)}$) and the rapid transient current ($I_{K(V)}$) recorded in medium containing 30 mM CaCl$_2$, but otherwise identical to that described in the legend to Figure 4. The outward currents were activated by depolarizing voltage step commands (20 to 70 mV in 10 mV jumps) from a holding potential of −60 mV. Voltage step commands to −40 mV and −30 mV activated the slow $I_{K(Ca)}$ which in high Ca$^{2+}$ was only slightly reduced by 100 μM baclofen. Voltage step commands to potentials between −20 mV and +10 mV activated both $I_{K(Ca)}$ and the predominant fast transient current $I_{K(V)}$. This combined outward current was markedly reduced by 100 μM baclofen.
were plotted as percentage reduction in peak \( I_{Ca} \) against the amplitude of the control peak \( I_{Ca} \) (Figure 9). The plot shows that with increasing amplitude of control \( I_{Ca} \) the current becomes more resistant to the action of baclofen.

**Discussion**

The results presented here suggest that baclofen reduced \( I_{Ca} \) by a direct rather than indirect action. \( I_{Ca} \) was recorded under conditions which minimized con-

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**Figure 8** The effect of 4-aminopyridine (4-AP) on the inward calcium current (\( I_{Ca} \)). In (a) the peak amplitude of \( I_{Ca} \) plotted against voltage achieved during the step command for control (C, ■), 4-AP (500 \( \mu \)M, ●) and recovery (R, ▲). The traces in (b) show maximum \( I_{Ca} \) under control conditions, in the presence of 4-AP and after recovery (1 of 5 experiments).

**Figure 9** The effect of 4-aminopyridine (4-AP) on the baclofen-induced inhibition of the inward calcium current (\( I_{Ca} \)). In (a), the % reduction of the peak amplitude of the maximum \( I_{Ca} \) by baclofen in different cells is plotted against the peak of the maximum control \( I_{Ca} \) in the same experiment. Plots show the effect of 100 \( \mu \)M baclofen alone (○) and 100 \( \mu \)M baclofen applied together with 500 \( \mu \)M 4-AP (▲). The lines were fitted by linear regression analysis. In (b) the traces show \( I_{Ca} \) reduced by baclofen alone and by baclofen applied together with 4-AP, in two different cells.
tamination by potassium conductances. However, TEA-insensitive potassium conductances have been well described in vertebrate neurones. For example, TEA did not block anomalous rectification of mammalian sympathetic ganglion cells (Christ & Nishi, 1973), adrenaline-induced hyperpolarizations in bullfrog sympathetic ganglion neurones (Koketsu & Nakamura, 1976) or the acetylcholine activated M-current (Brown & Adams, 1980). In addition, a recent study on K⁺ channels from mammalian sarcoplasmic reticulum in planar phospholipid bilayers has revealed that Cs⁺ is nearly as permeant as K⁺, although it is less conductive (Cukierman et al., 1985). For these reasons we examined the effect of another K⁺ channel blocker, 4-AP, since it has been shown to inhibit transient outward currents in a number of systems (Thompson, 1977; Siegelbaum & Tsien, 1980; Gustafson et al., 1982; Zbicz & Weight, 1985).

Application of 4-AP in this study induced a small increase in control ICa, suggesting that a residual TEA-insensitive outward K⁺ current was still present under the standard conditions for measuring ICa. To investigate further whether baclofen was producing a direct inhibition of ICa, baclofen was applied together with 4-AP with the result that the inhibition of ICa by baclofen was reduced. It is unlikely that this action of 4-AP could be accounted for by an effect on baclofen binding to its receptor, since 4-AP did not alter baclofen-displaceable [³H]-GABA binding to rat cortical membranes (personal communication, J.A. Cross).

Because of this finding, we next examined whether the effect of baclofen on ICa might be due to an enhancement of an underlying potassium current. However, baclofen decreased rather than increased both the calcium-dependent slow outward relaxation (ICa(Ca)), and the 4-AP sensitive fast transient outward current (ICa(Vo)), activated by depolarizing step commands. Thus these results do not explain why baclofen was a less effective inhibitor of ICa in the presence of 4-AP. It is likely that the inhibition by baclofen of ICa(Ca) reflects an inhibition of the underlying ICa, but the mechanism of its inhibition by ICa(Vo) is unknown, particularly since the current and its inhibition by baclofen remained in calcium-free medium, and 30 mM calcium medium.

The direct reduction by baclofen of ICa described here is consistent with other studies on rat and chick DRG neurones using GABA and baclofen (Dunlap, 1984; Deisz & Lux, 1985). An increase in outward current induced by baclofen has also been described (Newberry & Nicoll, 1985; Gähwiler & Brown, 1985), and probably represents a different mechanism by which GABA receptors modulate neuronal excitability occurs, and which is not present in cultured DRGs. The increase in K⁺ conductance induced by baclofen (Ibax) in hippocampal cells was blocked by external Ba²⁺ or internal Cs⁺ (Gähwiler & Brown, 1985), whereas it was under these conditions that ICa was reduced by baclofen in the present study. The two mechanisms of baclofen action may represent differences in presynaptic and postsynaptic GABAB receptor-effector mechanisms. Similarly, while adenosine receptor-mediated modulation of voltage-dependent inward calcium currents is present in cultured rat DRGs (Dolphin et al., 1985; 1986), it is not found in hippocampal pyramidal cells (Halliwell & Scholfield, 1984).

It is difficult to account for the observation that both baclofen and baclofen plus 4-AP induced a wide spread of inhibition of ICa in different cells. We observed that this inhibition tended to show an inverse correlation with the net amplitude of the control ICa. GABA-induced inhibition of ICa has also been found to be highly variable (Deisz & Lux, 1985). The simplest explanation for the percentage block by baclofen being smaller in cells with a larger control peak ICa would be some form of competition between the permeant divalent cation and baclofen, this competition being dependent on the number of calcium channels opening. In cardiac cells, calcium currents are inhibited by the organic calcium channel antagonists D600 (methoxyverapamil), diltiazem and nitrendipine, and the blocking action of these inhibitors is antagonized by increasing the external Ca²⁺ or Ba²⁺ concentration (Lee & Tsien, 1983). Although in the present study the dependence on external Ba²⁺ or Ca²⁺ of the baclofen inhibition of ICa was not determined, the inhibition by baclofen of ICa(Ca) was reduced when the external Ca²⁺ concentration was increased, and this presumably reflects a reduction in the effect of baclofen on ICa. It is also of interest that in ligand binding studies, the binding of agonists to GABAB sites is dependent on the presence of divalent cations (Bower et al., 1983).

A further consideration is that more than one type of calcium channel co-exist in neurones, and that as has been shown by Nowycky et al. (1985) these vary in their sensitivity to the calcium channel agonist Bay K 8644. Future work using whole cell recording and patch clamp techniques may reveal whether components of ICa in cultured rat DRGs have different sensitivities to baclofen and GABA, and the manner in which baclofen acts to produce its blockade of macroscopic Ca currents.

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References


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